

CLAIMS

- Claim 1. A method for preparing polynucleotide fragments for use in polynucleotide shuffling, comprising exposing at least one homologous heteroduplex polynucleotide to a polynucleotide repair system until said heteroduplex polynucleotide comprises at least one annealed fragment; and denaturing said heteroduplex polynucleotide to obtain said fragment.
- Claim 2. The method of claim 1, comprising fragmenting at least one strand of said heteroduplex polynucleotide by further exposing said heteroduplex polynucleotide to a DNase or restriction enzyme.
- Claim 3. The method of claim 1 or 2, wherein the steps occur *in vitro*.
- Claim 4. The method of claim 2, wherein said fragmenting comprises fragmenting with at least one restriction enzyme which has multiple cutting sites, or with a plurality of different restriction enzymes.
- Claim 5. The method of claim 4, wherein the fragments are at least 15 residues in length.
- Claim 6. The method of claim 1, wherein said heteroduplex polynucleotide is generated from a native gene by successive directed mutagenesis, by error-prone PCR, by random chemical mutagenesis, by *in vivo* random mutagenesis, or by combining genes from gene families within the same or different species.
- Claim 7. The method of claim 1, wherein said fragments are non-identical.
- Claim 8. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides and before exposing said heteroduplex polynucleotide to a polynucleotide repair system, promoting formation of said heteroduplex polynucleotide by

increasing the number of a parent polynucleotide in said library relative to other parent polynucleotides in said library.

- Claim 9. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides and before exposing said heteroduplex polynucleotide to a polynucleotide repair system, promoting formation of said heteroduplex polynucleotide by denaturing and rehybridizing the parent polynucleotides.
- Claim 10. The method of claim 1, wherein said polynucleotide repair system is a mismatch repair complex, a base excision repair complex, a nucleotide excision repair complex, phage T4 endonuclease VII, phage T7 endonuclease I, or a combination thereof.
- Claim 11. The method of claim 10, wherein said mismatch repair complex is DAM methylase, MutS, MutL, MutH, exonuclease, DNA helicase II, SSB protein, DNA polymerase III, DNA ligase, or a combination thereof.
- Claim 12. The method of claim 10, wherein said base excision repair complex is DNA glycosylase, AP endonuclease, DNA polymerase I, DNA ligase, or a combination thereof.
- Claim 13. The method of claim 10, wherein said nucleotide excision repair complex is Uvr-A, Uvr-B, Uvr-C, DNA polymerase I, DNA ligase, or a combination thereof.
- Claim 14. The method of claim 1, wherein exposing said heteroduplex polynucleotide to a polynucleotide repair system comprises incubating said parent polynucleotides with phage T4 endonuclease VII, phage T7 endonuclease I, or a combination thereof.
- Claim 15. The method of claim 4, wherein the fragments are about 15 residues in length to about X residues in length, wherein X equals one residue less

than the total number of residues in the longest polynucleotide in the reaction mixture.

- Claim 16. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides and before exposing said heteroduplex polynucleotide to a polynucleotide repair system, introducing at least one mismatch per parent polynucleotide.
- Claim 17. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides, and at least one strand of the parent polynucleotides is methylated.
- Claim 18. The method of claim 1, wherein said heteroduplex polynucleotide comprises dITP or uracil-containing DNA.
- Claim 19. The method of claim 1, wherein said heteroduplex polynucleotide comprises heteroduplex between DNA and RNA.
- Claim 20. The method of claim 1, wherein said polynucleotide repair system lacks polymerase, ligase or both.
- Claim 21. The method of claim 1, wherein said polynucleotide repair system only partially digests and partially cleaves mismatches.
- Claim 22. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides, and wherein at least one damaged base is introduced per initial parent polynucleotide.
- Claim 23. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides, and wherein at least one damaged nucleotide is introduced per initial parent polynucleotide.
- Claim 24. The method of claim 1, wherein the steps occur *in vivo*.

Claim 25. A polynucleotide shuffling reaction mixture comprising fragments of at least at two homologous heteroduplex polynucleotides, wherein said fragments were created at least in part by a polynucleotide repair system that lacked DNA polymerase.

Claim 26. A polynucleotide shuffling reaction mixture comprising fragments of at least two homologous heteroduplex polynucleotides for which damaged or mismatched residues have been repaired.

Claim 27. A polynucleotide shuffling reaction mixture comprising at least two homologous heteroduplex polynucleotides wherein at least one strand of said heteroduplex polynucleotides comprises fragments created at least in part by a polynucleotide repair system that lacked DNA polymerase.